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Membrane-bound Inclusions in the Leydig Cell Cytoplasm of the Broad-headed Skink, *Eumeces laticeps* (Lacertilia: Scincidae)

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ABSTRACT—The structure of the Leydig cell was studied in laboratory maintained skinks *Eumeces laticeps* (Schneider). Overall, skink Leydig cell cytology was similar to that of mammalian steroidogenic cells. Under both the light and electron microscope, for example, skink Leydig cells contained large lipid droplets in their cytoplasm. However, skink Leydig cells also contained elongated membrane bound rods and other rounded structures which were also surrounded by tightly packed concentric layers of membranes. The rounded structures were of two types, oval figures with empty interiors and fibrous structures with ribosome-like elements in their interiors. The elongatd, rod-like structures resembled crystalloids found in aging human corpora lutea. The oval structures resembled myelin sheaths and the round but wooly-looking bodies resembled compact whorls of membranes found in mice Leydig cells.

INTRODUCTION

A mammalian Leydig cell is often described as having extensive arrays of smooth endoplasmic reticulum appearing as long connected tubulues [3]. In the cytoplasm may be large lipid inclusions, which in some instances is evidence of cellular regression [1]. In addition, human and other mammalian Leydig cells, contain proteinaceous crystals of Reinke of unknown function or origin [8, 9]. Similarly, active Leydig cells in *Anolis* lizards, have extensive arrays of agranular endoplasmic reticulum, darkly-staining mitochondria and lipid granules without crystalline inclusions [13]. In the current study on the ultrastructure of skink Leydig cells, testes of laboratory maintained skinks *Eumeces laticeps* (Schneider) which were obtained during the periods of testicular recrudescence (January) and breeding (May) in the wild [7] were found to contain membrane-bound inclusions some of which looked crystal-like.

MATERIALS AND METHODS

Four animals captured in late winter around the campus were kept for 6 or more months in glass tanks, provided with water *ad libitum* and fed crickets daily. They were sacrificed either in January or May by decapitation with one stroke of scissors. Small pieces of testes were immersed in Karnovsky's fixative, pH 7.4 for 2 hr, postfixed in 1% OsO_4 in 0.1 M sodium cacodylate buffer at room temperature for 2 hr. After dehydration through alcohols and acetone they were embedded in Spurr's Epoxy mixture. Semi-thin sections were stained with toluidine blue and basic fuchsin (Electron Microscope Science) and ultrathin sections were stained with uranyl acetate and lead citrate and examined with a Philips 200 electron-microscope at an accelerating voltage of 60 kV.

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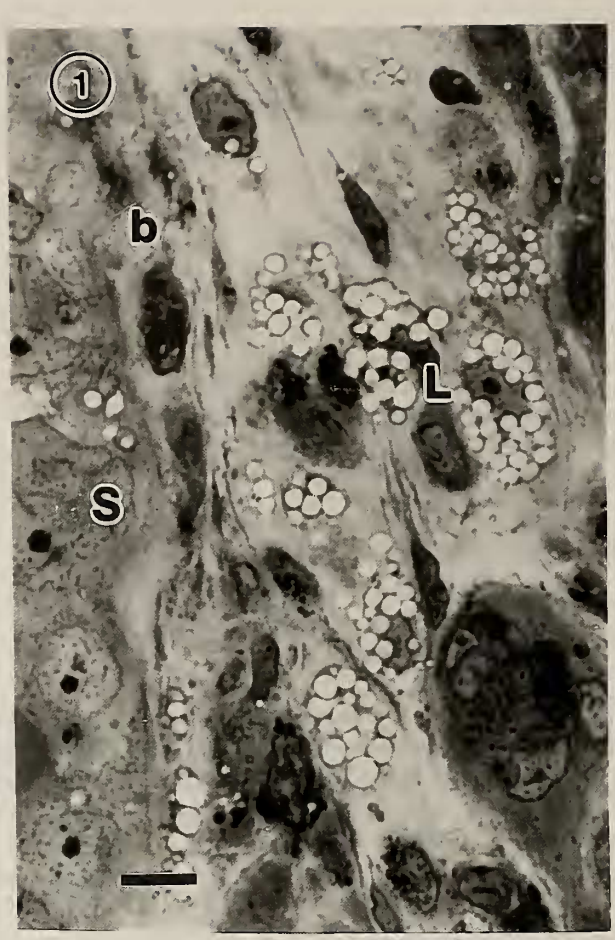
RESULTS

Under the light microscope, Leydig cells which were close to the basement membrane contained large lipid droplets in their cytoplasm (Fig. 1). Under the electron microscope, some of the Leydig cells similarly showed cytoplasms that were full of lipid granules with few other organelles visible (Fig. 2). The cytoplasm of what were considered Leydig cells was in all cases very granular and in some, contained inclusions consisting of rods which were visible at the level of the light microscope (Fig. 3). At the ultrastructural level, Leydig cell cytoplasm also contained other rounded structures that resembled myelin sheaths (Fig. 4). Between the rod-like structures and lipid granules were several free ribosomes and rough endoplasmic reticulum. Except for the solid dark outlines of the rods, their cores were not stained. The lipid granules on the other hand, had unstained cores and edges which were stained to varying degrees. Confluence between the lipid granules were sometimes observed (Fig. 5). Other than the simple rectangular pattern, some rods showed pointed ends and their darker outlines consisted of multiple layers of membranes (Fig. 6). Some rods contained fiber-like structures inside their cores (Fig. 7). At higher resolution, the dark walls of myelin-like structures and crystalloids consisted of multiple layers of membranes with alternating dark and light bands which resembled unit membranes (Figs. 8 and 9). In some sections, the light inner band between the 2 dark bands was bisected by a longitudinally positioned dark line (Fig. 10). The myelin structures sometimes enclosed portions of cytoplasm (Fig. 8) or empty spaces (Fig. 11). Also found was a wooly structure with whorls of concentric fiber enclosing ribosome-like elements (Fig. 12). All the observed structures were found in testes undergoing spermatogenesis and spermiogenesis and their shape or distribution was invariable in the sections examined.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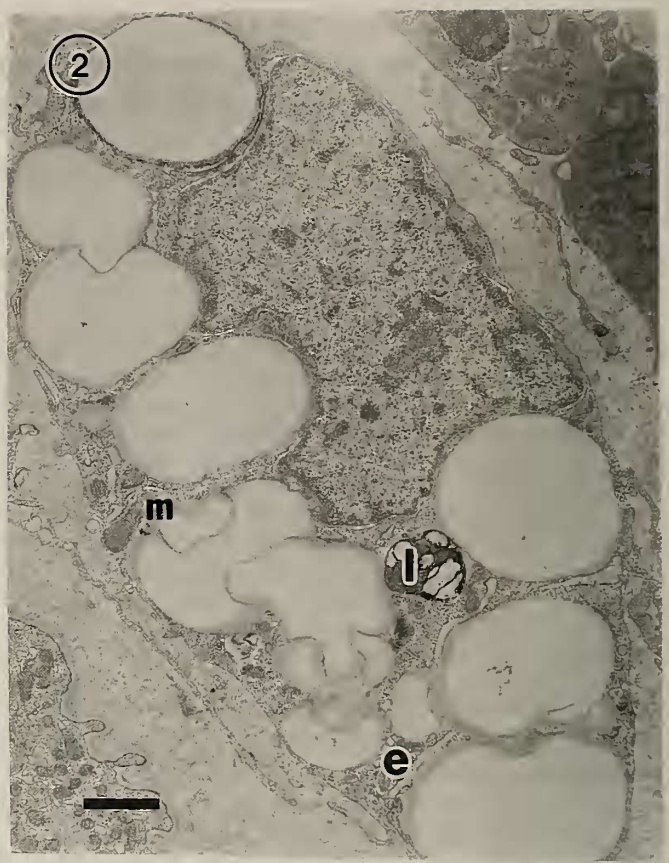
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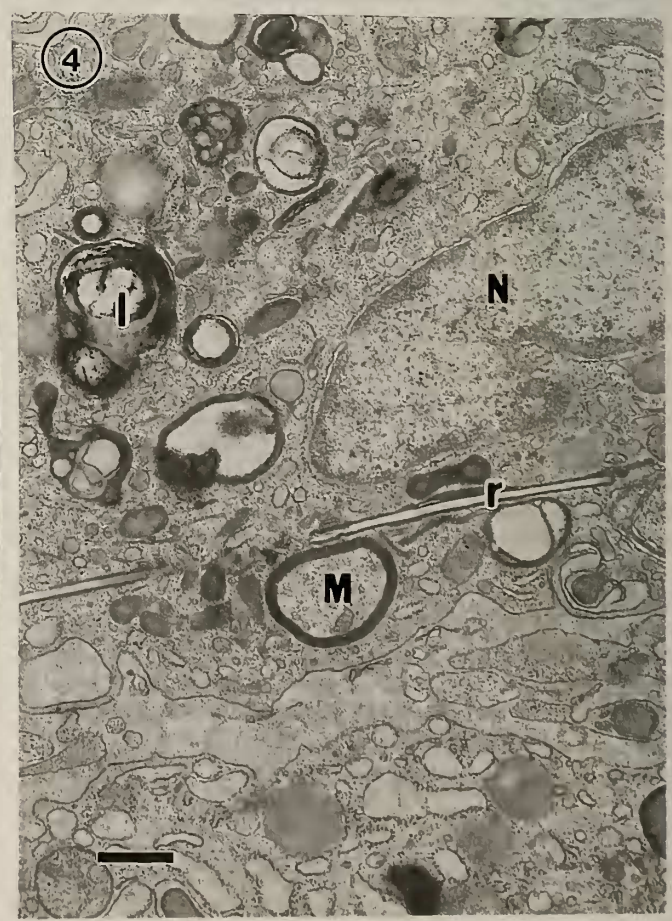
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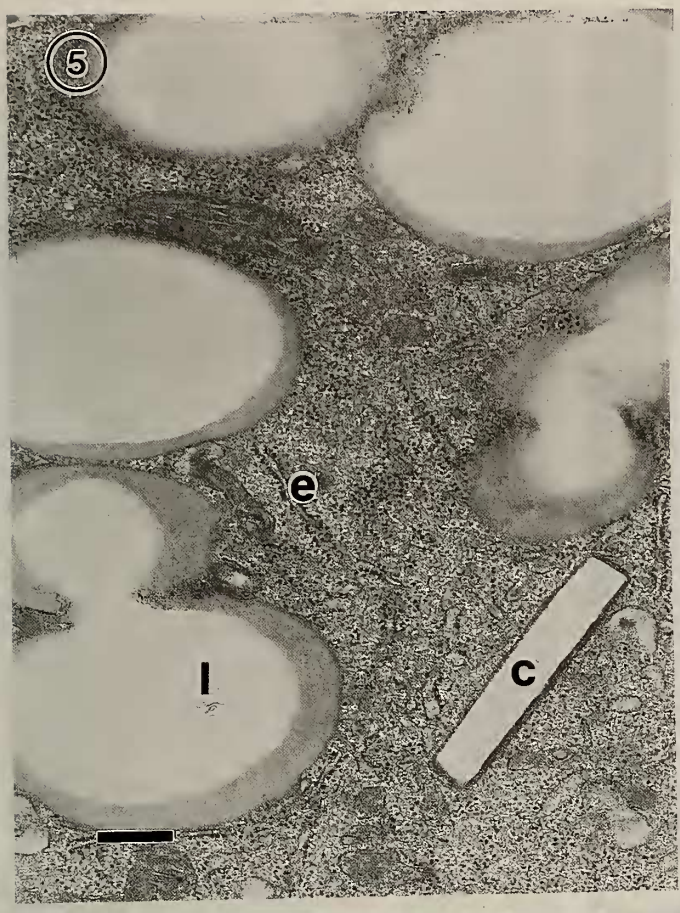
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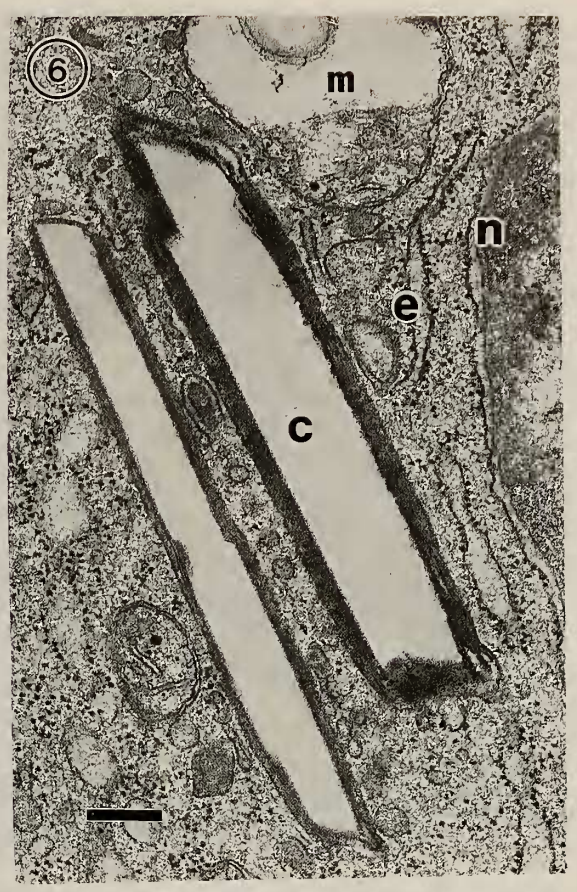
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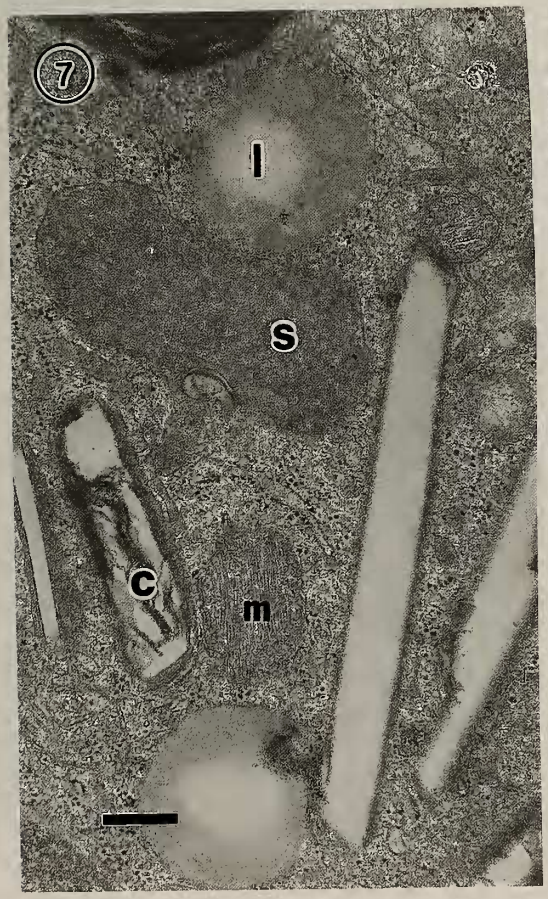
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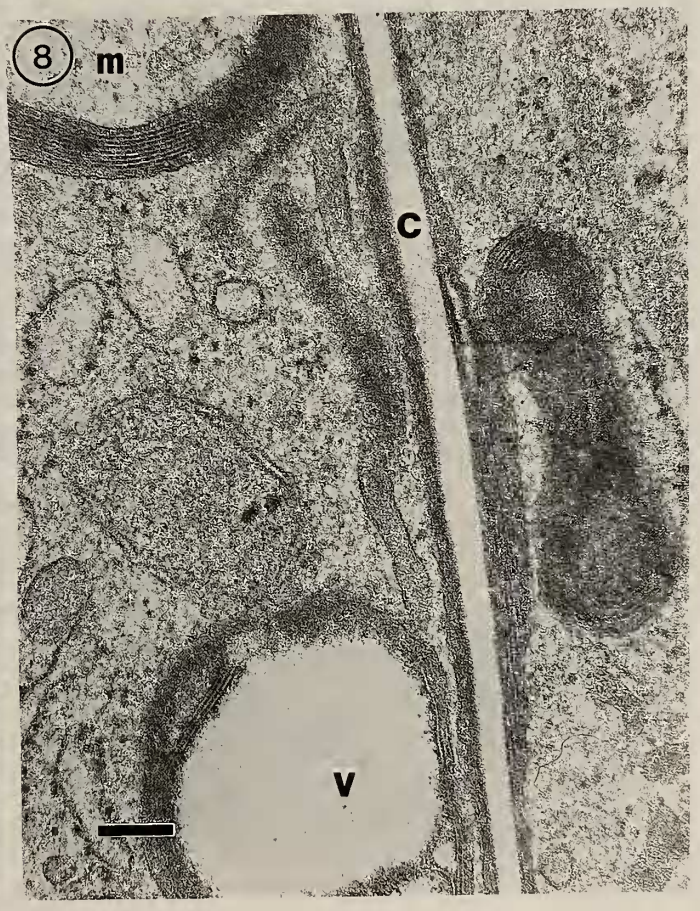
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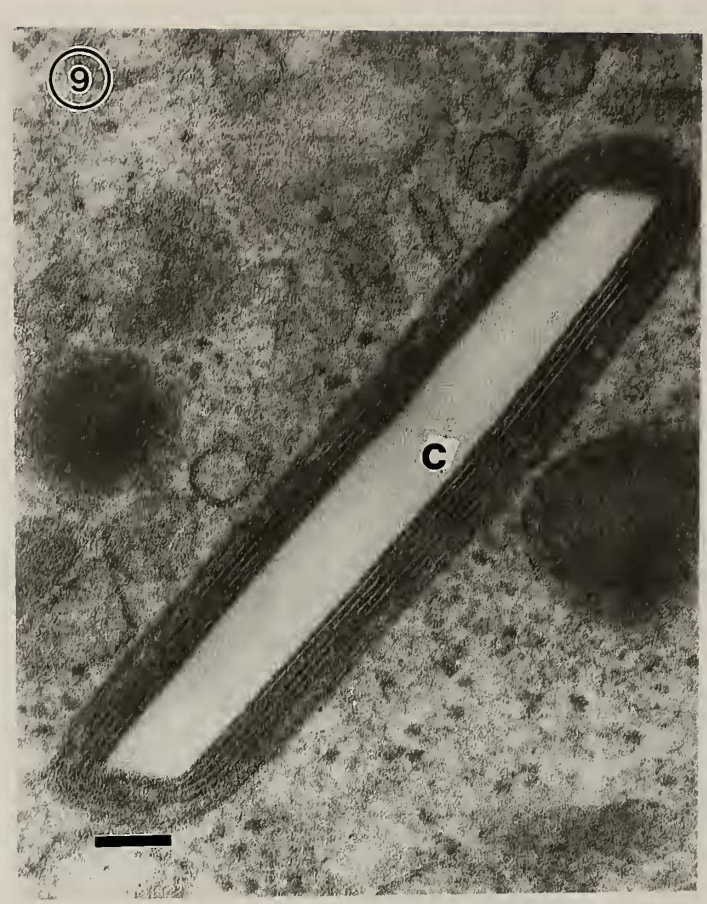
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DISCUSSION

A steroid producing cell should increase its membrane stores during active steroidogenesis and the increase in smooth endoplasmic reticulum in lipid producing cells is evidence of this. The ordered arrangement of smooth endoplasmic reticulum around liposomes in the armadillo Leydig cell is said to be one means of increasing surface area for cholesterol and steroid production [14]. Based on the appearance of their walls, it is possible that the inclusions found in skink Leydig cell cytoplasm are some special type of smooth endoplasmic reticulum like the kind described as tubular in the opossum Leydig cells [4]. However, only the rod-like inclusions in the skink Leydig cell cytoplasm can be considered tubular. The skink ovoid structures with laminated walls resemble lipid vacuoles found in the armadillo [14] and dog Leydig cells [6], which are surrounded by whorls of smooth endoplasmic reticulum. The skink membranes were however, more tightly packed and uniformly spaced like myelin figures in residual bodies [5]. Similarly, the inclusions with concentric whorls of membranes (Fig. 12) resemble those inclusions that have been described as "compact whorls of membranes" [8, 12] of unknown function rather than smooth endoplasmic reticulum. Therefore, these structures are not specialized smooth endoplasmic reticulum.

Crystals have been found in mammalian but not reptilian Leydig cells and so the data reported here are probably the first indication of crystal-like structures in reptilian Leydig cells. These rod-like structures in the skink Leydig cell cytoplasm resemble rod-like structures in aging human lutein cells [2, 5] described as lipid-soluble and possibly made of cholesterol. The characteristic of having laminated walls links them to cylindrical bodies that were described in rat Leydig cells [11] as having walls composed of a helical array of tubular units possibly derived from the endoplasmic reticulum [11]. But in the skink Leydig cell bodies, there was no connection between the membranes of the laminated walls and the endoplasmic reticulum. Though found in the Leydig cells, these skink rods do not resemble Reinke's crystals which occur in human and other mammalian Leydig cells. These bodies appear to be unique to the skink and their true nature has to await further investigation.

Like crystals, lipid droplets have also been found to increase in regressing Leydig cells [1, 10] and their occurrence in skink Leydig cells could be interpreted as portending their regression, but no evidence of such regression was noted.

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FIG. 1. The skink testis showing a cluster of Leydig cells (L) containing several lipid vesicles in the cytoplasm next to the basement membrane (b) of the seminiferous tubule, which shows four Sertoli cells (S) resting on the basement membrane. Bar=8 μm.

FIG. 2. A Leydig cell with large lipid granules occupying most of the cytoplasm. A few mitochondria (m), lysosome (I), and smooth endoplasmic reticulum (e) are also discernable. Bar=1 μm.

FIG. 3. A segment of the interstitial space showing Leydig cells located further from the basal lamina. The granularity of the cytoplasms and the presence of non-stained rods is evident (arrows). Bar=8 μm.

FIG. 4. This section through a Leydig cell shows a number of cytoplasmic inclusions including a myelin-like body (M), lysosome (I), non-stained rods (r). Also shown is the nucleus (N) with a ring of heterochromatin all along the inside of the nuclear envelope. Bar=0.6 μm.

FIG. 5. A section through a Leydig cell showing lipid granules (I), tubules of rough endoplasmic reticulum (e) and a non-stained rod or cylinder, here called a crystal (c). Bar: 0.3 μm.

FIG. 6. A Leydig cell cytoplasm showing two crystals (c) with their fibrous wall. An enlarged mitochondrion (m) with degenerating cristae is also shown. e, rough endoplasmic reticulum; n, nucleus. Bar=0.2 μm.

FIG. 7. The cytoplasm of a Leydig cell showing 4 crystalloids, one with a fibrous core (c); liposome (l), lysosome (s) and mitochondrion with laminated cristae (m). Bar=0.2 μm.

FIG. 8. A segment of a Leydig cell cytoplasm showing a number of membrane-bound inclusions all of which show evenly spaced parallel or concentric membranes. The figure is an enlargement of (Fig. 4). c, a crystal; m, myelin-like inclusion; v, vacuole. Bar=0.1 μm.

FIG. 9. The cytoplasm of a Leydig cells showing detail of the crystal (c) wall which consists of regularly spaced alternating light and dark bands. Bar=0.1 μm.

FIG. 10. A tangential section through a small crystal (c) in the cytoplasm of a Leydig cell, showing detail of the membranes making up the crystal wall. It shows that the light longitudinal band is bisected by another dark line (arrow). Bar=0.1 μm.

FIG. 11. A section through an oval shaped laminated structure within the cytoplasm of a Leydig cell. The wall of this structure consists of concentrically arranged membranes like those found around the crystalloids. Inside the structure are what appear to be cytoplasmic debris. Arrow points at the cell membrane for comparison with crystal wall membranes. Bar=0.1 μm.

FIG. 12. Another type of membrane-bound structure found in the cytoplasm of Leydig cells which resembles those structures that have been described in literature as "compact whorls of membranes". The wall of this structure encloses what looks like free ribosomes within the cytoplasm. Bar=0.2 μm.

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